CLAIMS

We claim:

1. An expression vector for producing IL-21 protein comprising the following operably linked elements:

(a) a prokaryotic origin of replication;

(b) a transcriptional initiation DNA element;

(c) a polynucleotide sequence as shown in SEQ ID NO:27; and

(d) a transcriptional terminator.

2. The expression vector of claim 1 which further comprises a selectable marker.

3. An expression vector comprising the pTAP337 vector, deposited with the American Type Culture Collection in Manassas, VA. under Patent Deposit Designation PTA-4853.

4. A prokaryotic host cell transformed with the expression vector according to claims 1, 2 or 3.

5. The host cell of claim 4, wherein the host cell is *E. coli* strain W3110.

6. A method for producing IL-21 proteins comprising:

(a) culturing a host cell according to claim 5 in growth medium under conditions wherein IL-21 is expressed;

(b) recovering the host cells from the growth medium; and

(c) isolating the IL-21 protein from the host cells.

7. A method for producing IL-21 proteins comprising:

(a) culturing a host cell according to claim 5 in growth medium by fed batch fermentation;

(b) recovering the host cells from the growth medium; and

(c) isolating the IL-21 protein from the host cells.

8. A method for producing an IL-21 protein comprising:

(a) culturing a host cell according to claim 4 or claim 5 in a shake flask to an OD600 of 5 to 20 in a growth medium;

(b) inoculating a fermentation vessel with 1 to 12% v/v of shake flask medium containing host cells;

(c) culturing the host cells in a growth medium at a pH of 6.2 to 7.2, wherein a feed solution is fed into the fermentation vessel before 15 hours elapsed fermentation time (EFT);

(d) adding an inducing agent to the fermentation vessel at 20 to 30 hours EFT; and

(e) harvesting the host cells at 48 to 56 hours EFT.

9. The method of claim 8, wherein the inducing agent is isopropyl thiogalactopyranoside (IPTG) at 0.5 to 2 mM.

10. The method of claim 8, wherein the feed solution comprises a carbohydrate selected from the group consisting of glycerol and glucose at a concentration of growth medium, and a feed rate of 5-15 grams of carbohydrate per hour.

11. The method of claim 10, wherein the glycerol is 40 to 70% v/v glycerol or the glucose is 40 to 70% w/v glucose.

12. The method of claim 10, wherein the glycerol is about 70% v/v or the glucose is about 60% w/v.

13. A method of producing IL-21 protein comprising:

(a) seeding a flask with an inoculum comprising an *E. coli* W3110 host cell expressing an IL-21 polypeptide as shown in SEQ ID NO:28, or an *E. coli* W3110 host cell comprising pTAP337 vector wherein an IL-21 polypeptide is expressed, and with growth medium comprising about 5 g/L glycerol;

(b) culturing the inoculum in growth medium for 16-20 hours at about 30°C;

(c) transferring the cultured inoculum in growth medium to a batch fermentor at a concentration of 0.5-5% v/v inoculum;

(d) fermenting the batch fermentation at about 37° and about pH 6.8; with about 2% glycerol;

(e) introducing a glucose feed at about 8 hours elapsed fermentation time (EFT) of about 9.5 g glucose/liter/hour and continuing until end of a fermentation run;

(f) adding IPTG at about 24 hour EFT to final concentration of 0.5 to 2 mM;

(g) fermenting about 28 hours after addition of IPTG;

(h) harvesting fermentation broth from the fermenter;

(i) adding an equal volume of water to the fermentation broth; and

(j) homogenizing and centrifuging the fermentation broth to collect a cell pellet or cell slurry comprising IL-21 protein material.

14. A method for isolating insoluble IL-21 protein comprising a sequence of amino acid residues as shown in SEQ ID NO:28 comprising the steps of:

(a) separating water insoluble IL-21 protein material from a cell pellet or cell slurry;

(b) dissolving the insoluble IL-21 protein material in a chaotropic solvent;

(c) diluting the chaotropic solvent and refolding the IL-21 protein; and

(d) isolating the IL-21 protein, wherein the isolated IL-21 protein is capable of being biologically active.

15. The method of claim 14 wherein the isolated IL-21 protein is at least 90% pure.

16. The method of claim 14 wherein the isolated IL-21 protein is at least 90% pure and has an endotoxin level of less than 10 endotoxin units per mg IL-21 protein.

17. A method for isolating insoluble IL-21 protein comprising a sequence of amino acid residues as shown in SEQ ID NO:28 comprising the steps of:

(a) separating from a fermentation broth a cell pellet or cell slurry comprising water insoluble IL-21 protein material;

(b) homogenizing the cell pellet or cell slurry to collect inclusion bodies;

(c) dissolving the insoluble IL-21 protein material in a chaotropic solvent comprising a guanidine salt;

(d) diluting the chaotropic solvent by addition of a refolding buffer comprising arginine salts and a mixture of reducing and oxiding components;

(e) isolating the IL-21 protein by removing unfolded and aggregated proteins by filtering; and

(f) purifying the IL-21 refolded protein on a cation exchange column; wherein the isolated and purified IL-21 protein is capable of being biologically active.

18. A method for isolating insoluble IL-21 protein comprising a sequence of amino acid residues as shown in SEQ ID NO:28 comprising the steps of:

(a) separating from a fermentation broth a cell pellet or cell slurry comprising water insoluble IL-21 protein material;

(b) homogenizing the cell pellet or cell slurry to collect inclusion bodies;

(c) dissolving the insoluble IL-21 protein material in a chaotropic solvent comprising a guanidine salt; and

(d) diluting the chaotropic solvent by addition of a refolding buffer comprising arginine salts and a mixture of reducing and oxidizing components;

(e) isolating the IL-21 protein by removing unfolded and aggregated proteins by filtering;

(f) purifying the IL-21 refolded protein on a cation exchange column; and

(g) purifying the IL-21 eluate from step (f) on a hydrophobic interaction column, wherein the isolated and purified IL-21 protein is capable of being biologically active.

19. A method for isolating insoluble IL-21 protein comprising a sequence of amino acid residues as shown in SEQ ID NO:28 comprising the steps of:

(a) separating from a fermentation broth a cell pellet or cell slurry comprising water insoluble IL-21 protein material;

(b) homogenizing the cell pellet or cell slurry to collect inclusion bodies;

(c) dissolving the insoluble IL-21 protein in a chaotropic solvent comprising about 6M guanidine hydrochloride, 40 mM dithiothreitol (DTT) for about one hour at room temperature;

(d) refolding the dissolved inclusion bodies in a solution by diluting into refolding buffer comprising about 0.75 M arginine, 2 mM DTT/4 mM cystine oxidation-reduction pair at least 20 times;

(e) adjusting pH to about 5.5 with about 20% acetic and allowing the solution to react for at least five hours;

(f) diluting the solution with about 1 + 1.4 volumes 25 mM acetate, pH 5.5;

(g) filtering the solution;

(h) loading solution on resin column equilibrated to pH 5.5 using sodium acetate buffer;

(i) washing the resin column with about 0.4 M sodium chloride;

(j) washing the resin column with about 0.75 M sodium chloride to elute bound IL-21 protein;

(k) adding ammonium sulfate to a concentration of about 1.5 M to eluate and filtering eluate solution;

(l) loading eluate onto a Tosohaas butyl 650-M column equilibrated to 1.5 M ammonium sulfate, 0.05 M sodium chloride in sodium acetate buffer;

(m) washing column with about 0.15 M ammonium sulfate, 0.05 sodium chloride in sodium acetate buffer;

(n) diluting the eluate to a conductivity of about 30 mS/cm with water;

(o) loading eluate onto a SP Sepharose HP column equilibrated with sodium acetate buffer;

(p) washing column with 20-column volume linear gradient from 0.3 to 0.7 M sodium chloride;

(q) concentrating the IL-21 protein; and

(r) exchanging buffer to formulation buffer using tangential flow ultrafiltration.

20. The method according to claims 13, 14, 15, or 16, wherein biological activity is measured using a IL-21 receptor-binding cell assay.

21. A composition comprising an IL-21 protein comprising amino acids residues 1-163 as shown in SEQ ID NO:28 at a concentration of about 10 mg/ml IL-21 protein in about 10 mM histidine, 4.7% mannitol at pH 5.3

22. A host cell from a strain of zGOLD1, deposited with the American Type Culture Collection (ATCC) in Manassas, VA transformed with an expression vector comprising a pTAP337 vector, deposited with the ATCC under Patent Deposit Designation PTA-4853.